

CLAIMS

WHAT IS CLAIMED IS:

1. An *ex vivo* method for selectively activating and expanding $\gamma\delta$ T cells in an isolated mixed cell population, the method comprising contacting the isolated mixed cell population with one or more soluble multivalent agents that selectively activate and expand $\gamma\delta$ T cells by binding to at least one epitope of a $\gamma\delta$ TCR, wherein the soluble multivalent agent is at least tetravalent.
2. An *ex vivo* method for selectively activating and expanding one or more $\gamma\delta$ T cell subtypes in an isolated mixed cell population, the method comprising contacting the isolated mixed cell population with one or more soluble multivalent agents that selectively activate and expand $\delta 1$ T cells, $\delta 2$ T cells, or $\delta 3$ T cells, or a combination thereof, preferably wherein the one or more agents that selectively activate and expand $\delta 1$ T cells bind to an activating epitope specific of a $\delta 1$ TCR; the one or more agents that selectively activate and expand $\delta 2$ T cells bind to an activating epitope specific of a $\delta 2$ TCR; and the one or more agents that selectively activate and expand $\delta 3$ T cells bind to an activating epitope specific of a $\delta 3$ TCR, thereby activating and expanding the desired $\gamma\delta$ T cell subtype in the mixed cell population, wherein the soluble multivalent agent is at least tetravalent.
3. An *in vitro* and/or *ex vivo* method for producing an enriched $\gamma\delta$ T-cell population comprising directly contacting an isolated mixed cell population comprising $\gamma\delta$ T-cells, or a purified fraction thereof, with one or more soluble multivalent agents; preferably wherein the soluble multivalent agent(s) activate and expand $\gamma\delta$ T cells by binding to at least one epitope of a $\gamma\delta$ TCR, wherein the soluble multivalent agent is at least tetravalent.
4. An *in vitro* and/or *ex vivo* method for producing enriched $\gamma\delta$ T-cell sub-populations from isolated mixed cell populations, comprising directly contacting an isolated mixed cell population with one or more soluble multivalent agents that i) selectively expand $\delta 1$ T-cells by binding to an epitope specific of a $\delta 1$ TCR, ii) that selectively expand $\delta 2$ T cells by binding to an epitope specific of a $\delta 2$ TCR, and iii) that selectively expand $\delta 3$ T cells by binding to an epitope specific of a $\delta 3$ TCR, to provide an enriched $\gamma\delta$ T cell sub-population, wherein the soluble multivalent agent is at least tetravalent.

5. The method according to any preceding claim, wherein the soluble multivalent agent comprises at least two antigen-binding sites that specifically bind the same antigen, or wherein the multivalent agent comprises at least two antigen-binding sites that specifically bind the same epitope of the same antigen.
6. The method according to any preceding claim, wherein the soluble multivalent agent is monospecific.
7. The method according to claim 5, wherein the antigen-binding sites bind to different epitopes on the constant or variable regions of δ TCR and/or γ TCR.
8. The method according to claim 5, wherein the antigen-binding sites comprise the CDRs from $\gamma\delta$ TCR pan MAbs, preferably wherein the antigen-binding sites specifically bind domains shared by different γ and δ TCRs on either the γ or δ chain or both, including $\delta 1$, $\delta 2$, and $\delta 3$ T cell populations.
9. The method according to claim 5, wherein the soluble multivalent agent selectively expands $\delta 1$ T-cells and comprises at least two, or greater than two, antigen-binding sites that specifically bind to an epitope comprising a $\delta 1$ variable region.
10. The method according to claim 9, wherein the soluble multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind a $\delta 1$ TCR Bin 1 $\delta 1$ epitope, Bin 1b $\delta 1$ epitope, Bin 2 $\delta 1$ epitope, Bin 2b $\delta 1$ epitope, Bin 2c $\delta 1$ epitope, Bin 3 $\delta 1$ epitope, Bin 4 $\delta 1$ epitope, Bin 5 $\delta 1$ epitope, Bin 6 $\delta 1$ epitope, Bin 7 $\delta 1$ epitope, Bin 8 $\delta 1$ epitope, or a Bin 9 $\delta 1$ epitope of a human $\delta 1$ TCR.
11. The method according to claim 9, wherein the soluble multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind to the same, or essentially the same, epitope as, or competes with, an antibody selected from the group consisting of $\delta 1$ -05, $\delta 1$ -08, $\delta 1$ -18, $\delta 1$ -22, $\delta 1$ -23, $\delta 1$ -26, $\delta 1$ -35, $\delta 1$ -37, $\delta 1$ -39, $\delta 1$ -113, $\delta 1$ -143, $\delta 1$ -149, $\delta 1$ -155, $\delta 1$ -182, $\delta 1$ -183, $\delta 1$ -191, $\delta 1$ -192, $\delta 1$ -195, $\delta 1$ -197, $\delta 1$ -199, $\delta 1$ -201, $\delta 1$ -203, $\delta 1$ -239, $\delta 1$ -253, $\delta 1$ -257, $\delta 1$ -278, $\delta 1$ -282, and $\delta 1$ -285.

12. The method according to claim 9, wherein the soluble multivalent agent comprises the CDRs of an antibody selected from the group consisting of δ 1-05, δ 1-08, δ 1-18, δ 1-22, δ 1-23, δ 1-26, δ 1-35, δ 1-37, δ 1-39, δ 1-113, δ 1-143, δ 1-149, δ 1-155, δ 1-182, δ 1-183, δ 1-191, δ 1-192, δ 1-195, δ 1-197, δ 1-199, δ 1-201, δ 1-203, δ 1-239, δ 1-253, δ 1-257, δ 1-278, δ 1-282, and δ 1-285.
13. The method of claim 9, wherein the soluble multivalent agent comprises the CDRs of antibody δ 1-35 or δ 1-08, or binds the same epitope as antibody δ 1-08 or δ 1-35.
14. The method according to claim 9, wherein the soluble multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind the same epitope as an antibody selected from TS-1 and TS8.2.
15. The method according to claim 14, wherein the soluble multivalent agent comprises the CDRs of TS-1 or TS8.2 and/or is a humanized TS-1 or TS8.2.
16. The method according to claim 9, wherein the soluble multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind to an epitope comprising residues Arg71, Asp72 and Lys120 of the δ 1 variable region.
17. The method according to claim 5, wherein the soluble multivalent agent selectively expands δ 1 T-cells and δ 3 T cells.
18. The method according to claim 5, wherein the soluble multivalent agent selectively expands δ 1 T cells selectively expands δ 1, δ 3, δ 4, and δ 5 $\gamma\delta$ T cells.
19. The method according to claim 5, wherein the soluble multivalent agent selectively expands δ 2 T-cells and comprises at least two, or greater than two, antigen-binding sites that specifically bind to an epitope comprising a δ 2 variable region.
20. The method according to claim 19, wherein the soluble multivalent agent comprises at least two, or greater than two, antigen-binding sites that have reduced binding to a mutant δ 1 TCR polypeptide comprising a mutation at K120 of delta J1 and delta J2.

21. The method according to claim 19, wherein the soluble multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind a $\delta 2$ TCR Bin 1 $\delta 2$ epitope, Bin 2 $\delta 2$ epitope, Bin 3 $\delta 2$ epitope, or Bin 4 $\delta 2$ epitope of a human $\delta 2$ TCR.
22. The method according to claim 19, wherein the soluble multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind to the same, or essentially the same, epitope as, or competes with, an antibody selected from the group consisting of $\delta 2$ -14, $\delta 2$ -17, $\delta 2$ -22, $\delta 2$ -30, $\delta 2$ -31, $\delta 2$ -32, $\delta 2$ -33, $\delta 2$ -35, $\delta 2$ -36, and $\delta 2$ -37.
23. The method according to claim 22, wherein the soluble multivalent agent comprises the CDRs of an antibody selected from the group consisting of $\delta 2$ -14, $\delta 2$ -17, $\delta 2$ -22, $\delta 2$ -30, $\delta 2$ -31, $\delta 2$ -32, $\delta 2$ -33, $\delta 2$ -35, $\delta 2$ -36, and $\delta 2$ -37.
24. The method of claim 19, wherein the soluble multivalent agent comprises the CDRs of antibody $\delta 2$ -37 or binds the same epitope as antibody $\delta 2$ -37.
25. The method according to claim 19, wherein the soluble multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind to the same epitope as an antibody selected from 15D and B6.
26. The method according to claim 24, wherein the soluble multivalent agent comprises the CDRs of antibody 15D or B6 and/or is a humanized 15D and B6 antibody.
27. The method according to claim 19, wherein the soluble multivalent agent comprises at least two, or greater than two, antigen-binding sites that have reduced binding to a mutant $\delta 2$ TCR polypeptide comprising a mutation at G35 of the $\delta 2$ variable region.
28. The method according to claim 5, wherein the soluble multivalent agent selectively expands $\delta 3$ T-cells and comprises at least two, or greater than two, antigen-binding sites that specifically bind to an epitope comprising a $\delta 3$ variable region.
29. The method according to claim 28, wherein the soluble multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind to the same, or essentially the

same, epitope as, or competes with, an antibody selected from the group consisting of $\delta 3$ -08, $\delta 3$ -20, $\delta 3$ -23, $\delta 3$ -31, $\delta 3$ -42, $\delta 3$ -47 and $\delta 3$ -58.

30. The method according to claim 29, wherein the soluble multivalent agent comprises the CDRs of an antibody selected from the group consisting of $\delta 3$ -08, $\delta 3$ -20, $\delta 3$ -23, $\delta 3$ -31, $\delta 3$ -42, $\delta 3$ -47 and $\delta 3$ -58.

31. The method according to any preceding claim, wherein the method comprises simultaneously or sequentially culturing the $\gamma\delta$ T-cell population with a cytokine, preferably wherein the cytokine is a common gamma chain cytokine.

32. The method according to claim 31, wherein the cytokine is selected from the group consisting of IL-2, IL-7, IL-9, IL-12, IL-15, IL-18, IL-19, IL-21, IL 23, and IL-33, preferably wherein the cytokine is IL-2, IL-15, IL-12, or IL-21.

33. The method according to any preceding claim, wherein the $\gamma\delta$ T-cell population is engineered before and/or after activation and/or expansion.

34. The method according to claim 33, wherein the $\gamma\delta$ T-cell population is engineered to stably express one or more tumor recognition moieties encoded by expression cassettes, and/or wherein the $\gamma\delta$ T-cell population is engineered to stably express a transgene that encodes at least one secreted cytokine.

35. The method according to claim 34, wherein the at least one secreted cytokine is a common gamma chain cytokine selected from the group consisting of IL-2, IL-15 and IL-4, preferably wherein the cytokine is IL-2, IL-15 or IL-4.

36. A soluble composition comprising one or more multivalent agent(s) for selectively activating and expanding $\gamma\delta$ T cells, a subtype thereof, or combinations thereof, wherein said multivalent agent(s) comprises at least two antigen-binding sites that specifically bind the same epitope of a $\gamma\delta$ TCR; preferably wherein the multivalent agent is at least tetravalent.

37. The soluble composition according to claim 36, wherein the multivalent agent is monospecific.
38. The soluble composition according to claim 36, wherein the antigen-binding sites comprise the CDRs from $\gamma\delta$ TCR pan MAbs, preferably wherein the multivalent agent comprises the amino acid sequence set forth in Fig. 20 or 23.
39. The soluble composition according to claim 36, wherein the multivalent agent selectively expands $\delta 1$ T-cells and comprises at least two, or greater than two, antigen-binding sites that specifically bind to an epitope comprising a $\delta 1$ variable region.
40. The soluble composition according to claim 39, wherein the multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind a $\delta 1$ TCR Bin 1 $\delta 1$ epitope, Bin 1b $\delta 1$ epitope, Bin 2 $\delta 1$ epitope, Bin 2b $\delta 1$ epitope, Bin 2c $\delta 1$ epitope, Bin 3 $\delta 1$ epitope, Bin 4 $\delta 1$ epitope, Bin 5 $\delta 1$ epitope, Bin 6 $\delta 1$ epitope, Bin 7 $\delta 1$ epitope, Bin 8 $\delta 1$ epitope, or a Bin 9 $\delta 1$ epitope of a human $\delta 1$ TCR.
41. The soluble composition according to claim 39, wherein the multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind to the same, or essentially the same, epitope as, or competes with, an antibody selected from the group consisting of $\delta 1$ -05, $\delta 1$ -08, $\delta 1$ -18, $\delta 1$ -22, $\delta 1$ -23, $\delta 1$ -26, $\delta 1$ -35, $\delta 1$ -37, $\delta 1$ -39, $\delta 1$ -113, $\delta 1$ -143, $\delta 1$ -149, $\delta 1$ -155, $\delta 1$ -182, $\delta 1$ -183, $\delta 1$ -191, $\delta 1$ -192, $\delta 1$ -195, $\delta 1$ -197, $\delta 1$ -199, $\delta 1$ -201, $\delta 1$ -203, $\delta 1$ -239, $\delta 1$ -253, $\delta 1$ -257, $\delta 1$ -278, $\delta 1$ -282, and $\delta 1$ -285.
42. The soluble composition according to claim 39, wherein the multivalent agent comprises the CDRs of an antibody selected from the group consisting of $\delta 1$ -05, $\delta 1$ -08, $\delta 1$ -18, $\delta 1$ -22, $\delta 1$ -23, $\delta 1$ -26, $\delta 1$ -35, $\delta 1$ -37, $\delta 1$ -39, $\delta 1$ -113, $\delta 1$ -143, $\delta 1$ -149, $\delta 1$ -155, $\delta 1$ -182, $\delta 1$ -183, $\delta 1$ -191, $\delta 1$ -192, $\delta 1$ -195, $\delta 1$ -197, $\delta 1$ -199, $\delta 1$ -201, $\delta 1$ -203, $\delta 1$ -239, $\delta 1$ -253, $\delta 1$ -257, $\delta 1$ -278, $\delta 1$ -282, and $\delta 1$ -285.
43. The soluble composition of claim 39, wherein the multivalent agent comprises the CDRs of antibody $\delta 1$ -35 or $\delta 1$ -08, or binds the same epitope as antibody $\delta 1$ -08 or $\delta 1$ -35, preferably wherein the multivalent agent comprises the amino acid sequence set forth in Fig. 1 or 2.

44. The soluble composition according to claim 39, wherein the multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind the same epitope as an antibody selected from TS-1 and TS8.2.
45. The soluble composition according to claim 44, wherein the soluble multivalent agent comprises the CDRs of TS-1 or TS8.2 and/or is a humanized TS-1 or TS8.2.
46. The soluble composition according to claim 39, wherein the multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind to an epitope comprising residues Arg71, Asp72 and Lys120 of the $\delta 1$ variable region.
47. The soluble composition according to claim 36, wherein the multivalent agent selectively expands $\delta 1$ T-cells and $\delta 3$ T cells.
48. The soluble composition according to claim 36, wherein the multivalent agent selectively expands $\delta 1$ T cells selectively expands $\delta 1$, $\delta 3$, $\delta 4$, and $\delta 5$ $\gamma\delta$ T cells.
49. The soluble composition according to claim 36, wherein the multivalent agent selectively expands $\delta 2$ T-cells and comprises at least two, or greater than two, antigen-binding sites that specifically bind to an epitope comprising a $\delta 2$ variable region.
50. The soluble composition according to claim 49, wherein the multivalent agent comprises at least two, or greater than two, antigen-binding sites that have reduced binding to a mutant $\delta 1$ TCR polypeptide comprising a mutation at K120 of delta J1 and delta J2.
51. The soluble composition according to claim 49, wherein the multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind a $\delta 2$ TCR Bin 1 $\delta 2$ epitope, Bin 2 $\delta 2$ epitope, Bin 3 $\delta 2$ epitope, or Bin 4 $\delta 2$ epitope of a human $\delta 2$ TCR.
52. The soluble composition according to claim 49, wherein the multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind to the same, or essentially the same, epitope as, or competes with, an antibody selected from the group consisting of $\delta 2$ -14, $\delta 2$ -17, $\delta 2$ -22, $\delta 2$ -30, $\delta 2$ -31, $\delta 2$ -32, $\delta 2$ -33, $\delta 2$ -35, $\delta 2$ -36, and $\delta 2$ -37.

53. The soluble composition according to claim 52, wherein the multivalent agent comprises the CDRs of an antibody selected from the group consisting of δ 2-14, δ 2-17, δ 2-22, δ 2-30, δ 2-31, δ 2-32, δ 2-33, δ 2-35, δ 2-36, and δ 2-37.
54. The soluble composition of claim 49, wherein the multivalent agent comprises the CDRs of antibody δ 2-37 or binds the same epitope as antibody δ 2-37.
55. The soluble composition according to claim 49, wherein the multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind to the same epitope as an antibody selected from 15D and B6.
56. The soluble composition according to claim 55, wherein the multivalent agent comprises the CDRs of antibody 15D or B6 and/or is a humanized 15D and B6 antibody.
57. The soluble composition according to claim 49, wherein the multivalent agent comprises at least two, or greater than two, antigen-binding sites that have reduced binding to a mutant δ 2 TCR polypeptide comprising a mutation at G35 of the δ 2 variable region.
58. The soluble composition according to claim 36, wherein the multivalent agent selectively expands δ 3 T cells and comprises at least two, or greater than two, antigen-binding sites that specifically bind to an epitope comprising a δ 3 variable region.
59. The soluble composition according to claim 58, wherein the multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind to the same, or essentially the same, epitope as, or competes with, an antibody selected from the group consisting of δ 3-08, δ 3-20, δ 3-23, δ 3-31, δ 3-42, δ 3-47 and δ 3-58.
60. The soluble composition according to claim 59, wherein the multivalent agent comprises the CDRs of an antibody selected from the group consisting of δ 3-08, δ 3-20, δ 3-23, δ 3-31, δ 3-42, δ 3-47 and δ 3-58.
61. Use of a soluble composition according to any one of claims 36-60 in the manufacture of a medicament for treating a cancer, infectious disease, inflammatory disease, or an autoimmune

disease in a subject in need thereof, wherein the medicament comprises the resulting expanded $\gamma\delta$ T cell population, subtype thereof, or admixtures thereof.